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Abstract: BACKGROUND Tamoxifen is frequently used for the treatment of hormone receptor positive breast cancer (BC). Mainly CYP2D6 is responsible for the transformation to therapeutically active metabolites, but CYP2C19, CYP2C9 and CYP2B6 also are involved. We investigated the impact of polymorphisms within the genes encoding these CYP enzymes on the relapse-free time (RFT) in patients with BC. METHODS Ninety-nine patients with hormone receptor positive BC, who had undergone adjuvant tamoxifen therapy, were genotyped for seventeen common variants within the genes encoding CYP2D6, CYP2C9, CYP2C19 and CYP2B6 using TaqMan and PCR-RFLP technology. Kaplan-Meier and Cox regression analyses were performed to elucidate the impact of genetic variants on RFT. Furthermore, CYP2D6 metabolic activity was determined in a subset of 50 patients by assessing dextromethorphan/dextrorphan urinary excretion ratios. CYP2D6 activity was compared to the CYP2D6 allelic combinations to evaluate the predictive value of the CYP2D6 genotyping results on phenotype. RESULTS Although a trend toward longer RFTs in carriers of CYP2D6 allele combinations encoding for extensive and ultrafast metabolizer phenotypes was observed, none of the investigated genetic variants had a statistically significant impact on RFT. The combined analysis of five major CYP2D6 variants was useful for the discrimination between poor and non-poor metabolizers. CONCLUSIONS Comprehensive CYP2D6 genotyping has a good predictive value for CYP2D6 activity. Common variants in CYP2C9, CYP2C19, CYP2D6, and CYP2B6 did not have a significant impact on the RFT in this cohort of patients with BC.

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Impact of variable CYP Genotypes on Breast Cancer Relapse in Patients undergoing adjuvant Tamoxifen Therapy

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Abstract

Background: Tamoxifen is frequently used for the treatment of hormone receptor positive breast cancer (BC). Mainly CYP2D6 is responsible for the transformation to therapeutically active metabolites, but CYP2C19, CYP2C9 and CYP2B6 also are involved. We investigated the impact of polymorphisms within the genes encoding these CYP enzymes on the relapse free time (RFT) in breast cancer patients.

Methods: Ninety-nine hormone receptor positive breast cancer patients, who had undergone adjuvant tamoxifen therapy, were genotyped for seventeen common variants within the genes encoding *CYP2D6*, *CYP2C9*, *CYP2C19*, and *CYP2B6* using TaqMan and PCR/RFLP technology. Kaplan-Meier and Cox regression analyses were performed to elucidate the impact of genetic variants on RFT. Furthermore, CYP2D6 metabolic activity was determined in a subset of 50 patients by assessing dextromethorphan/dextrorphan urinary excretion ratios. CYP2D6 activity was compared to the *CYP2D6* allelic combinations to evaluate the predictive value of the CYP2D6 genotyping results on phenotype.

Results: Although a trend towards longer RFTs in carriers of *CYP2D6* allele combinations encoding for extensive and ultrafast metabolizer phenotypes was observed, none of the investigated genetic variants had a statistically significant impact on RFT. The combined analysis of five major *CYP2D6* variants was useful for the discrimination between poor and non-poor metabolizers.

Conclusions: Comprehensive CYP2D6 genotyping has a good predictive value for CYP2D6 activity. Common variants in *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP2B6* did not have a significant impact on the RFT in this cohort of BC patients.

Abbreviations

AS, activity score; BC, breast cancer; DFS, disease free survival; DM, Dextrometorphan; DX, Dextrorphan; ER, estrogen receptor; EDTA, ethylenediaminetetraacetic acid; EM, extensive metabolizer; HPLC, high-pressure liquid chromatography; IM, intermediate metabolizer; PM, poor metabolizer; PR, progesterone receptor; PCR, polymerase chain reaction; RFT, relapse free time; STD, standard deviation; UM, ultrafast metabolizer; 4-OH-tamoxifen, 4-hydroxytamoxifen

Key words:

breast cancer, tamoxifen, drug metabolism, genetic polymorphisms, therapy efficacy, relapse free time

Introduction

Breast cancer (BC) is one of the most frequent cancers in the western world. Tamoxifen, a selective estrogen receptor modulator (SERM), is widely used as an efficient therapeutic option in the treatment and relapse prevention of estrogen receptor-positive breast cancer [12]. Tamoxifen is transformed predominantly by the drug metabolizing enzymes CYP3A4 and CYP2D6 into the therapeutically more efficient drug metabolites 4-hydroxytamoxifen (4-OH-tamoxifen) and endoxifen. By binding to the estrogen receptor α (ER α) tamoxifen and its metabolites modulate the estrogen-induced transcription of ER α target genes. The metabolites 4-OH-tamoxifen and endoxifen show up to 100 times higher affinity to the estrogen receptor α (ER α) than the parental compound [7]. As a result, the efficacy of tamoxifen strongly depends on its appropriate bioactivation by cytochrome P450 enzymes. CYP2D6 is highly polymorphic and shows a high interindividual variability in its activity. Currently, there are more than 100 genetic variants described for *CYP2D6* [21]. Several genetic variants are known to cause an impaired enzyme activity (*CYP2D6**10, *41) or to even induce a total loss of enzyme function (e.g. *CYP2D6**4, *5, *6). Furthermore, gene duplications or multiplications of *CYP2D6* that occur in 2-3% of white individuals may lead to an exceptionally fast metabolism of CYP2D6 substrates. Genetic polymorphisms of *CYP2D6* have been repeatedly associated with a changed therapeutic outcome in BC patients treated with tamoxifen. Several studies could demonstrate that genetic variants causing an impaired CYP2D6 enzyme activity or even a loss of CYP2D6 function are associated with a shorter relapse free time (RFT) upon tamoxifen therapy [10, 20]. Other studies, however, showed conflicting results. Especially in studies where the efficacy of tamoxifen in relation to the CYP2D6 metabolizer status was tested in an adjuvant setting, no clear relationship between CYP2D6 status and therapeutic outcome was observed [16, 18]. The latter studies are, however, controversial in the literature, due to the facts that DNA was isolated from tumor tissues and not from blood, and that Hardy-Weinberg equilibria were partly not reached for several *CYP2D6* genetic variants [14, 23]. Other enzymes involved in tamoxifen metabolism comprise CYP2C9, CYP2C19, and CYP2B6 [13]. These three enzymes are also involved in the formation of 4-OH-tamoxifen and endoxifen, but their contribution may depend on actual tamoxifen concentrations and on CYP2D6 activity.

In this study, we studied the impact of genetic variants in the genes encoding the drug metabolizing enzymes CYP2C9, CYP2C19, CYP2B6 and CYP2D6 on the RFT in BC patients treated with tamoxifen in an adjuvant setting. We chose to investigate genetic variants that have been demonstrated to have a significant impact on the metabolic efficacy of the enzymes in focus in order to evaluate to which extent these polymorphisms might be suitable to predict the therapeutic outcome with tamoxifen.

Patients and Methods

Patients

Ninety-nine patients were included in this study. Patients were of Caucasian origin, diagnosed with early-stage estrogen receptor (ER)-positive BC and treated with adjuvant tamoxifen. Patients were recruited at the Breast Center Zurich, Switzerland between July 2009 and December 2012. Tumors were demonstrated to be ER positive in all patients and 91% had a >20% ER expression on the surface. The majority of cases also exhibited PR. Patients were treated with tamoxifen alone or sequentially with aromatase inhibitors (AIs) as the primary treatment choice. In case of a chemotherapeutic treatment (75.8%), regimens comprised the combinations FEC (5-fluorouracil, epirubicin and cyclophosphamide), FEC-TXT (5-fluorouracil, epirubicin, cyclophosphamide and docetaxel) or EC-TAX (epirubicin, cyclophosphamide and paclitaxel). Data of primary BC diagnosis and disease recurrence were confirmed from the patients' pathological and medical records. The study was approved by the local ethical committee of Zurich, and written informed consent was obtained. The demographic and disease-associated characteristics of all patients included in the study are shown in table 1.

Genotyping

From all patients, a whole blood sample was collected in EDTA vials. Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. Extracted DNA was used to genotype all patients for 17 genetic variants within the genes encoding cytochrome P450 enzymes involved in the metabolism of tamoxifen. The genotyping comprised the allelic variants *CYP2C9**2 and *3, *CYP2C19**2, *3, *4, *6 and *17, *CYP2B6**6, *7 and *16, *CYP2D6**3, *4, *5, *6, *10 and *41 as well as analysis of *CYP2D6* gene duplications/multiplications. The majority of variants were determined using TaqMan SNP assay technology (Agilent Technologies, Santa Clara, CA, USA). The variant *CYP2D6**41 was analyzed using a conventional nested PCR approach followed by digestion of the PCR product with the restriction enzyme BsePI. The forward primer used in the second PCR reaction carried an artificial mutation in the area of the 3' end, thereby creating a functional restriction site for BsePI in interplay with the wild type form at the *CYP2D6**41 locus. The determination of *CYP2D6* duplications/multiplications was performed as described previously [24].

Genotypes of *CYP2D6* were assigned to the expected corresponding metabolizer phenotypes according to the evaluation system by Gaedigk et al. [8]. The genotype/phenotype assignments are summarized in table 2.

CYP2D6 phenotyping and CYP2D6 metabolizer type assignment

Phenotypes predicted on the basis of *CYP2D6* genotypes were compared to the CYP2D6 phenotypes measured in a subset of BC patients. Phenotypic data were obtained by High Pressure Liquid Chromatography (HPLC) according to the methods described by Abdel-Rahman et al. [1] and Blake et al. [3]. HPLC based phenotype was determined as follows: Six hours after the intake of 25mg dextrometorphan (DM), a prototypical CYP2D6 substrate, the concentrations of methyl-dextrophan (MD) and its O-demethylated metabolite dextrophan (DX) were determined in the urine of 50 patients by reversed-phase HPLC with fluorescence detection. For this purpose, 1.25ml of each urine sample was deglucuronidated by adding 500ul phosphate buffer and 25ul glucuronidase. The samples were incubated at 50°C, cooled down, and subsequently centrifuged and included in the further analysis. The DM/DX ratios were calculated using the peak height concentrations of DM and DX, determined by HPLC. Patients were considered as CYP2D6 phenotypic PM, when exhibiting a DM/DX excretion ratio of > 0.3 , as described previously [4, 25]. Furthermore, patients were evaluated as CYP2D6 phenotypic IM, EM or UM, when showing DM/DX metabolic ratios of $0.03 - 0.3$, $0.0003 - (<0.03)$, and <0.0003 , respectively.

Statistical analysis

The chi-square test and the Fisher's exact test were used to compare categorical variables, such as e.g. the dichotomized response measure in relation to the abundance of different genetic variants (e.g. genotype frequencies) or any pathological molecular markers within the patient groups. Continuous data (e.g. age or duration of tamoxifen/AI intake) are given as median \pm STD or mean plus time range and were compared using the Mann-Whitney U test. The Kruskal-Wallis test was performed to compare the observed DM/DX ratios in the predicted CYP2D6 phenotype groups.

The relapse free time (RFT) was determined in patients who were taking tamoxifen for at least 12 months. The RFT was defined as the time interval in months between the time point of BC diagnosis and the occurrence of the first relapse of disease. A relapse was defined as the occurrence of an event such as either metastatic disease, local or distant recurrence of the tumor, or a new invasive primary breast cancer. The RFT was estimated using the Kaplan-Meier method and statistically analyzed using the log-rank test. A two-sided p-value of < 0.05 was considered statistically significant. The impact of any putative confounders on RFT, such as tumor stage, chemotherapy, menopausal or ER status was tested performing a Cox regression analysis. The program SPSS (version 21, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

Ninety-nine patients were genotyped for 17 different polymorphisms in the genes encoding the drug metabolizing enzymes CYP2C9, CYP2C19, CYP2D6 and CYP2B6 using leukocytic DNA. In case of *CYP2D6*, seven different genetic variants were determined, including gene duplications/multiplications and the most important variants (*3, *4, *5, *6, *10, *41) responsible for an impaired enzyme activity or the expression of null alleles. With the exception of the mutation *CYP2C19**6, all variants were in Hardy-Weinberg-equilibrium. The variant *CYP2C19**6 appeared only once in homozygous form in the cohort and was therefore calculated to be in Hardy-Weinberg-dysequilibrium.

Fifteen patients developed a relapse of breast cancer within the observational period. Table 1 demonstrates and compares the demographic and disease characteristics of the individuals showing a relapse with the patient group that stayed disease free during the time of observation. In general, no significant differences were observed when comparing both groups with regard to age, disease stage, grade, or menopausal status. A relapse was significantly less frequently observed in patients with > 20% ER expression ($p < 0.001$, chi-square test). The observational period and, consequently, the average time of tamoxifen intake was significantly longer in the relapse free group ($p < 0.001$ and $p = 0.003$, respectively).

Eighty-six patients were successfully genotyped for *CYP2D6* duplications/multiplications and could, thus, be grouped according to their predicted CYP2D6 phenotypes based on the calculated activity scores for CYP2D6 ([9], table 2). As demonstrated in table 2 no significant differences were detected in the overall frequencies of predicted CYP2D6 phenotypes in relation to the relapse status. Additionally, genotype based predicted CYP2D6 phenotypes were correlated with the experimentally confirmed phenotype (urinary DM/DX metabolic ratio) in a subgroup of 50 patients, where both the genotypic and phenotypic information was available. As demonstrated in figure 1 the metabolic DM/DX ratios differed significantly between the predicted CYP2D6 phenotypes ($p = 0.001$, Kruskal-Wallis test). The prediction of phenotypes based on genotyping results was especially well reproducible for the discrimination between poor and non-poor metabolizers (table 3). A sensitive discrimination between intermediate, extensive and ultrafast metabolizers was, however, only possible to a limited extent (figure 1, table 3).

All ninety-nine patients were included into the analysis investigating, to which extent the genotypes of *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP2B6* influence the relapse time in BC patients treated with tamoxifen. In several sub analyses, the estrogen receptor status, the menopausal state and a concomitant chemotherapeutic treatment were considered additional putative risk factors and were also taken into consideration. In the case of ER status, it was assumed that an expression degree of > 20% would be an important prerequisite for an efficient therapy

approach with antiestrogens, such as tamoxifen. The average duration of tamoxifen intake was 30 months (range of 12 to 77 months) and aromatase inhibitors 10 months (range of 0 to 64 months). The median follow-up time of patients was 46 months. To analyze the influence of genetic variants detected in *CYP2C9*, *CYP2C19*, *CYP2B6*, and *CYP2D6* genes on the BC-RFT the following approach was adopted. First, the frequencies of variant carriers (heterozygous plus homozygous individuals) were compared in the relapse and relapse free groups before (table 4) and after a logistic regression analysis stratifying for ER density, menopausal state, disease grade and stage and an additional chemotherapeutic treatment. Second, the RFTs were analyzed in relation to the investigated genotypes using the Kaplan-Maier method without and with stratification for ER and menopausal status (Cox regression analysis). No significant associations were observed between *CYP2C9*, *CYP2C19* and *CYP2B6* genotypes and the risk for a BC relapse before or after stratification for the different putative confounders. The ER state appeared to be an independent risk factor for the development of a disease relapse: 6 of 9 patients with an ER expression below or equal to 20% had a relapse in comparison to 9 of 90 with an ER expression above 20% ($p < 0.001$). As demonstrated in figure 2 a trend towards a lower relapse rate was observed for individuals carrying allelic combinations that induce an EM or UM genotype of *CYP2D6* ($p = 0.19$). This trend was repeatedly observed, when stratifying for different risk confounders.

Discussion

Several studies could demonstrate that interindividual differences in the activity of CYP2D6 may affect the therapeutic outcome in breast cancer patients receiving tamoxifen in an adjuvant setting [19, 20]. Besides CYP2D6, other drug-metabolizing enzymes play a role in the *in vivo* transformation of tamoxifen, but were, in this context, rarely investigated. Therefore, our aim was to study to which extent genetic polymorphisms in *CYP2D6*, *CYP2C9*, *CYP2C19*, and *CYP2B6* either alone or in interplay with each other influence the relapse risk under treatment with tamoxifen in a cohort of Caucasian breast cancer patients.

Although not significant, we could show in concordance with previous studies [19, 20] that genotypes associated with normal or increased CYP2D6 activity lead to a favorable treatment outcome under tamoxifen, when taking the re-occurrence of BC as a primary endpoint. This trend towards lower relapse rates can be explained by the increased production of the potent metabolites 4-OH-tamoxifen and endoxifen. The variant *CYP2C19**17 has been associated with a fast metabolizer phenotype of CYP2C19 due to an increased expression of CYP2C19 [2, 22]. *CYP2C19**17 may, thus, lead to an augmented production of 4-OH-tamoxifen, which may improve the treatment outcome as reported by Schroth et al. 2007 [19]. In our study, however, we did not observe an effect of *CYP2C19**17 on treatment outcome, neither when analyzing the impact of the *17 variant on the relapse rate alone nor in combination with fast metabolizer genotypes of *CYP2D6*.

Different genotypes of *CYP2C9*, *CYP2C19* or *CYP2B6* did not significantly alter the risk for a relapse of BC under treatment with tamoxifen, which underlines the stronger impact of CYP2D6 on tamoxifen pharmacokinetics. Of note, we used DNA from peripheral blood mononuclear cells (PBMCs) for our analysis, which more accurately reflects the DNA sequence of CYP genes in the liver, whereas DNA obtained from e.g. tumor samples may harbor additional cancer-induced mutations, as has been recently discussed [17].

Although several meta-analyses demonstrated that patients with CYP2D6 extensive metabolizer genotypes/phenotypes as compared to slow metabolizer geno- and phenotypes have a longer disease-free survival, the overall survival appeared not to be significantly influenced by the CYP2D6 metabolizer state in tamoxifen-treated BC patients. Furthermore, these studies provided inconclusive results with regard to potential treatment guidelines for tamoxifen based on CYP2D6 genotyping results. [11, 15, 26].

Instead of assigning four metabolizer phenotypes to genotypes, we tested the application of the CYP2D6 genotyping evaluation system of Gaedigk et al. [9], which weighs the impact of different *CYP2D6* genotypes on CYP2D6 enzyme activity by assigning activity scores (AS) to the allelic combinations. By correlating predicted to observed CYP2D6 phenotypes, we could demonstrate that the AS system, if based on comprehensive *CYP2D6* genotyping results, appears to be a useful approach to discriminate between slow and extensive

metabolizers. Phenotypic poor and intermediate metabolizers were especially well predicted. A finer discrimination especially between intermediate, extensive and ultrafast metabolizers is, however, not reliably possible based on this system. Since not all possible genetic variations in *CYP2D6* were investigated in our study, misclassification may also arise from the assignment of the **1* allele to individuals who harbor a rare, activity-lowering *CYP2D6* mutation. Additionally, *CYP2D6* phenotyping by using urinary dextromethorphan-based metabolite ratios may not be the optimal phenotyping tool, because small activity differences cannot be reliably detected [5, 6], which in turn, may also lead to misclassifications.

Besides an estrogen-receptor expression below 20%, no other factor like demographics, menopausal state or chemotherapy regimen used were identified as a risk for a relapse.

Our study includes a limited number of individuals and was performed in a retrospective manner. Both aspects could potentially weaken the associations between genotypes and therapeutic outcome with tamoxifen especially in cases, where a polymorphism might have a weak impact on the breast cancer recurrence risk. The data reported here should be validated in the future by e.g. the performance of prospective studies assessing the impact of *CYP2D6* variants on the therapeutic outcome of BC in large cohorts of tamoxifen treated BC patients.

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Conflict of interest

All authors declare that there are no conflicts of interest

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Table 1. Demographic information and disease characteristics of patients treated with tamoxifen for the treatment of breast carcinoma (% in parentheses unless stated otherwise)

	All (n = 99)	No relapse (n = 84)	Relapse (n = 15)	<i>p</i> value ^{a)}
Age (years, median, range)	48 (28-77)	48 (28-73)	50 (37-77)	ns
Stage				
I-IIa	65 (65.7)	57 (67.9)	8 (53.3)	ns
IIb-IIIc	34 (34.3)	27 (32.1)	7 (46.7)	
ER > 20%	90 (90.9)	81 (96.4)	9 (60.0)	<0.001
PR presence	84 (84.8)	75 (89.3)	9 (60.0)	ns
HER-2 positive	22 (22.2)	21 (25.0)	1 (6.7)	ns
Grade				
1	10 (10.1)	9 (10.7)	1 (6.7)	
2	46 (46.5)	40 (47.6)	6 (40.0)	ns
3	43 (43.4)	35 (41.7)	8 (53.3)	
Observational period (months (min-max))	47.8 (12-130)	51.6 (12-130)	26.5 (12-72)	<0.001 ^{b)}
Tamoxifen intake (months (min-max))	30.4 (12-77)	32.3 (12-77)	19.9 (12-50)	0.003 ^{b)}
AI intake (months (min-max))	10.3 (0-64)	9.8 (0-50)	12.9 (0-64)	ns ^{b)}
Premenopause (%)	47 (47.5)	40 (47.6)	7 (46.7)	ns
Chemotherapy (%)	75 (75.8)	63 (75.0)	12 (80.0)	ns

^{a)} Fisher's exact tests or chi-square tests were performed.

^{b)} The Mann-Whitney U test was performed

Table 2. Prediction of CYP2D6 enzyme activity based on *CYP2D6* genotyping results and the assignment of activity scores (AS) according to Gaedigk et al. [8, 9]

Designation	AS value assigned to the allelic combinations ^{a)}	Genotypes ^{b)}	All n = 98 (%)	No relapse n = 83 (%)	Relapse n = 15 (%)	p value ^{c)}
Poor metabolizer (PM)	0	*4/*4, *6/*6	6 (6.7)	5 (6.0)	1 (6.7)	
Intermediate metabolizer (IM)	0.5	*3/*10, *4/*10, *6/*10 *4/*41, *6/*41,	33 (33.7)	27 (32.5)	6 (40.0)	
	1	*1/*3, *1/*4, *1/*5, *1/*6, *10/*10, *10/*41, *41/*41	9 (9.2)	8 (9.6)	1 (6.7)	ns
	1.5	*1/*10, *1/*41	20 (20.4)	18 (21.7)	2 (13.3)	
Extensive metabolizer (EM)	2	*1/*1	25 (25.5)	22 (26.5)	3 (20.0)	
Ultrarapid metabolizer (UM)	>2	Duplications or multiplications of functional alleles	5 (5.1)	3 (3.6)	2 (13.3)	

^{a)} AS assignment to alleles:

*3, *4, *5, *6 (non functional), AS 0; *10, *41, AS 0.5 (reduced function); *1, AS 1 (wild type allele); duplication/multiplication, AS 2 (increased function)

^{b)} only observed genotypes are listed

^{c)} The chi-square test was applied

Table 3. Concordance of predicted and measured CYP2D6 phenotypes in a subset of BC patients

Genotype based predicted CYP2D6 phenotype	Patients (n = 50) n (%)	Concordance Predicted/observed	Observed phenotype (based on DM/DX ratio)	
			Median	Range
PM	3 (6)	3/4	1.803	1.490 – 5.337
IM	36 (72)	36/32	0.097	0.004 – 0.433
EM	8 (16)	8/13	0.016	0.000 – 0.090
UM	3 (6)	3/1	0.021	0.016 – 0.130

Table 4. Genotype frequencies of *CYP2C9*, *CYP2C19*, *CYP2B6* and *CYP2D6* before and after stratification for disease behavior in 99 BC patients

Gene	Allele	Genotype, n ^{a)} (%), all patients			Genotype, n (%), relapse free group			Genotype, n (%), relapse group			p-value (variant vs. non variant carrier)
		wt	het	hom	wt	het	hom	wt	het	hom	
<i>CYP2C9</i>	*2	76 (76.8)	22 (22.2)	1 (1.0)	65 (77.4)	18 (21.4)	1 (1.2)	11 (73.3)	(4) 26.7	0 (0.0)	ns
	*3	85 (85.9)	13 (13.1)	1 (1.0)	70 (83.3)	13 (15.5)	1 (1.2)	15 (100)	(0) 0.0	1 (1.0)	ns
<i>CYP2C19</i>	*2	72 (72.7)	22 (22.2)	5 (5.1)	60 (71.4)	20 (23.8)	4 (4.8)	12 (80.0)	(2) 13.3	1 (6.7)	ns
	*3	99 (100.0)	0 (0.0)	0 (0.0)	84 (100.0)	0 (0.0)	0 (0.0)	15 (100.0)	(0) 0.0	0 (0.0)	ns
	*4	98 (99.0)	1 (1.0)	0 (0.0)	83 (98.8)	1 (1.2)	0 (0.0)	15 (100.0)	(0) 0.0	0 (0.0)	ns
	*6	98 (99.0)	0 (0.0)	1 (1.0)	83 (98.8)	0 (0.0)	1 (1.2)	15 (100.0)	(0) 0.0	0 (0.0)	ns
	*17	67 (67.7)	28 (28.3)	4 (4.0)	57 (67.9)	23 (27.4)	4 (4.8)	10 (66.7)	(5) 33.3	0 (0.0)	ns
<i>CYP2B6</i>	*6	54 (54.5)	40 (40.4)	5 (5.1)	46 (54.8)	34 (40.5)	4 (4.8)	8 (53.3)	(6) 40.0	1 (6.7)	ns
	*7	80 (82.5)	17 (17.5)	0 (0.0)	68 (82.9)	14 (17.1)	0 (0.0)	80 (82.5)	(17) 17.5	0 (0.0)	ns
	*16	98 (99.0)	1 (1.0)	0 (0.0)	83 (98.8)	1 (1.2)	0 (0.0)	15 (100.0)	(0) 0.0	0 (0.0)	ns
<i>CYP2D6</i>	*3	96 (98.0)	2 (2.0)	0 (0.0)	81 (97.6)	2 (2.4)	0 (0.0)	15 (100.0)	(0) 0.0	0 (0.0)	ns
	*4	60 (61.2)	34 (34.7)	4 (4.1)	52 (62.7)	28 (33.7)	3 (3.6)	8 (53.3)	(6) 40.0	1 (6.7)	ns
	*5	97 (98.0)	2 (2.0)	0 (0.0)	83 (98.8)	1 (1.2)	0 (0.0)	14 (93.3)	(1) 6.7	0 (0.0)	ns
	*6	93 (94.9)	4 (4.1)	1 (1.0)	78 (94.0)	4 (4.8)	1 (1.2)	15 (100.0)	(0) 0.0	0 (0.0)	ns
	*10	52 (53.1)	39 (39.8)	7 (7.1)	44 (53.0)	34 (41.0)	5 (6.0)	8 (53.3)	(5) 33.3	2 (13.3)	ns
	*41	75 (79.8)	18 (19.1)	1 (1.1)	64 (80.0)	15 (18.8)	1 (1.3)	11 (78.6)	(3) 21.4	0 (0.0)	ns
	Dupl./Multipl. ^{b)}	77 (89.5)	9 (10.5)		66 (90.4)	7 (9.6)		11 (84.6)	2 (15.4)		ns

^{a)} n denotes the absolute number of individuals carrying the respective genotype

^{b)} In each subgroup the abundance of *CYP2D6* gene duplications/multiplications is compared (left column: not abundant, right column: abundant)

Figure Legends

Figure 1. CYP2D6 phenotypes were predicted on the basis of CYP2D6 activity scores, as calculated from *CYP2D6* genotyping results. Predicted CYP2D6 phenotypes were correlated with measured CYP2D6 phenotypes, determined on the basis of urinary DM/DX ratios. 50 patients were included, where both genotype and phenotype data were available. The plot represents median values with data ranges. The Kruskal-Wallis test detected significant differences of DM/DX ratios between the different AS score groups ($p = 0.001$).

Figure 2. Kaplan-Meier analyses determining and comparing the RFT in months in individuals carrying *CYP2D6* slow metabolizer (PMs plus IMs) and fast metabolizer genotypes (EMs plus UMs), *CYP2C19* wild type and *CYP2C19**17, or *CYP2B6* wild type and *CYP2B6**6. Although not significant, a trend towards a more favourable disease outcome was observed for carriers of genotypes coding for fast metabolizer phenotypes ($p = 0.19$). The comparisons in case of *CYP2C19* and *CYP2B6* polymorphisms were not significant (p ns).

Fig 1

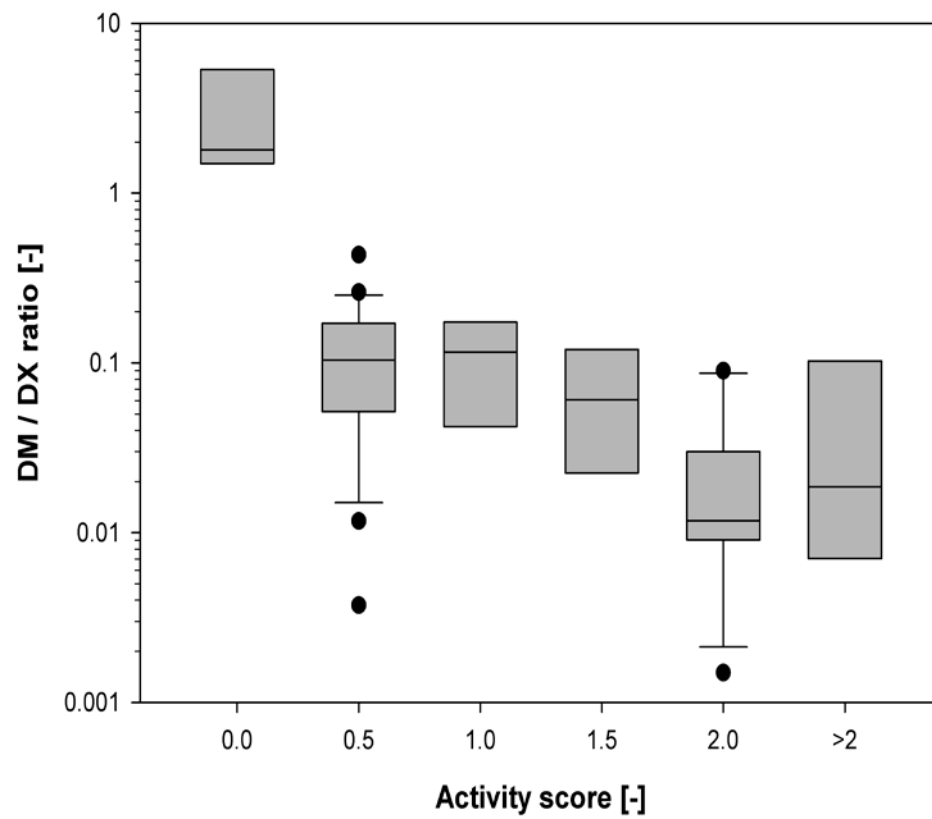


Fig 2

